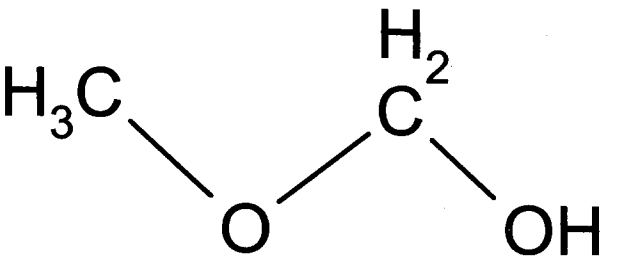
201-15973B

# Methoxymethanol

## CAS Number 4461-52-3






# HPV Data Set

| | | |
|---|---|---|
| **Existing Chemical** | : | ID: 4461-52-3 |
| **CAS No.** | : | 4461-52-3 |
| **EINECS Name** | : | methoxymethanol |
| **EC No.** | : | 224-722-2 |
| **Molecular Formula** | : | C2H6O2 |

**Producer related part**

**Company** : Celanese Ltd
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| | | |
|---|---|---|
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### 1.0.1 APPLICANT AND COMPANY INFORMATION

| | | |
|---|---|---|
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23.12.2003

### 1.2 SYNONYMS AND TRADENAMES

**Formaldehyde methyl hemiacetal**

20.08.2003

**Hemiformal**

20.08.2003

**Methanol, hemiformal**

20.08.2003

**Methanol, methoxy- (8CI9CI)**

20.08.2003

**Methyl hemiformal**

20.08.2003

## 2.1 MELTING POINT

**Remark** :
There is no defined melting/freezing point for this mixture.
At temperatures below 65 deg. C, solid polymeric formaldehyde gradually forms.
At temperatures below 0 deg. C, ice crystals can form

In the environment the material will readily dissociate to:
Formaldehyde with a melting point of -92 deg. C (Merck Index, 13th Edition)
Methanol with a melting point of -97.8 deg C (Merck Index, 13th Edition)

**Test substance** :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

**Reliability** : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

**Flag** : Critical study for SIDS endpoint
22.11.2003 (8)

## 2.2 BOILING POINT

**Value** : ca. 90 - 95 °C at 1013 hPa

**Remark** :
The boiling point will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid

In the environment the material will readily dissociate to:
Formaldehyde with a boiling point of -19.5 deg. C @1013 hPa (Merck Index, 13th Edition)
Methanol with a boiling point of 64.7 deg. C @1013 hPa (Merck Index, 13th Edition)

**Test substance** :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

**Reliability** : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

**Flag** : Critical study for SIDS endpoint
22.11.2003 (8)

## 2.3 DENSITY

## 2.4 VAPOUR PRESSURE

**Value** : ca. 90 - 95 hPa at 40 °C

**Result** :

The vapor pressure will vary depending on the exact composition of the mixture. The range given is for the specified mixture. As other compositions of this mixture may be sold, this range may not be universally valid.

In the environment the material will readily dissociate to:

Formaldehyde with a vapor pressure of 5174 hPa @ 25 deg. C (Boublik, T., Fried, V., and Hala, E., The Vapour Pressures of Pure Substances. Second Revised Edition. Amsterdam: Elsevier, 1984. 44 as cited in HSDB)

Methanol with a vapor pressure of 169 hPa @ 25 deg. C (Boublik T et al; The vapor pressures of pure substances: selected values of the temperature dependence of the vapour pressures of some pure substances in the normal and low pressure region. Vol. 17. Amsterdam, Netherlands: Elsevier Sci. Publ 1984. as cited in HSDB)

**Test substance** :

Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

**Reliability** : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a variable mixture

**Flag** : Critical study for SIDS endpoint

22.11.2003 (8)

## 2.5 PARTITION COEFFICIENT

**Partition coefficient** : octanol-water
**Log pow** : ca. -1.4 at 25 °C
**pH value** :
**Method** : other (calculated)
**Year** :
**GLP** :
**Test substance** :

**Method** :

Calculated using EPIWIN 3.05 using SMILES input of COCO

**Remark** :

In the environment the material will readily dissociate to:

Formaldehyde with a log Kow of 0.35 (Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995. 3, as cited in HSDB)

Methanol with a log Kow of -0.77 (ibid.)

**Test substance** :

Methoxymethanol CASNO 4461-52-3, assumed pure

**Reliability** : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

**Flag** : Critical study for SIDS endpoint
22.11.2003 (3)

### 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

**Solubility in** : Water
**Value** : at °C
**pH value** :
**concentration** : at °C
**Temperature effects** :
**Examine different pol.** :
**pKa** : at 25 °C
**Description** :
**Stable** :

**Remark** :
EPIWIN predicted water solubility of pure material is >1000g/L (EPIWIN 3.05 calculation using SMILES of COCO)

In the environment the material will readily dissociate to:

Formaldehyde with a water solubility >1000g/L (Merck Index, 13th Edition)
Methanol with a a water solubility >1000g/L (Merck Index, 13th Edition)

**Result** :
Miscible

**Test substance** :
Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water.

**Reliability** : (2) valid with restrictions

Reliability assigned as 2 since this is experimental data on a water reactive mixture.

**Flag** : Critical study for SIDS endpoint
22.11.2003 (8)

### 3.1.1 PHOTODEGRADATION

| | | |
|---|---|---|
| **Type** | : | air |
| **Light source** | : | Sun light |
| **Light spectrum** | : | nm |
| **Relative intensity** | : | based on intensity of sunlight |
| **Spectrum of substance** | : | lambda (max, >295nm) : nm |
| | | epsilon (max) : |
| | | epsilon (295) : 0 |
| **DIRECT PHOTOLYSIS** | | |
| **Halflife t1/2** | : | > 1 year |
| **Degradation** | : | % after |
| **Quantum yield** | : | |
| **INDIRECT PHOTOLYSIS** | | |
| **Sensitizer** | : | OH |
| **Conc. of sensitizer** | : | 1500000 molecule/cm³ |
| **Rate constant** | : | cm³/(molecule*sec) |
| **Degradation** | : | > 50 % after 15.8 hour(s) |
| **Deg. product** | : | |
| **Method** | : | other (calculated): APOWIN |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | other TS: Mixture |

**Method** :

As this equilibrium mixture nominally contains methoxymethanol, formaldehyde, methanol and water, and since the initial content of methoxymethanol will be rapidly converted to formaldehyde and methanol, calculations were conducted independently for the three main components.

As there was a discrepancy between the theoretical value of the rate constant for reaction of formaldehyde with hydroxyl radical and an experimental value obtained by Atkinson in 1994, the AOPWIN program was also run on hydrated formaldehyde, which is considered to be in equilibrium with formaldehyde in atmospheres containing water.

**Result** :

DIRECT PHOTOLYSIS

None of these materials has a chromophore with significant absorption above 295 nm, therefore, direct photolysis is not considered to be an important process in the fate of methoxymethanol preparations.

INDIRECT PHOTOLYSIS

The results of the calculations are shown below. The experimentally derived rate constant for reaction of formaldehyde with hydroxyl radical (Atkinson, 1994) is reconciled by it being a combined rate constant of formaldehyde and hydrated formaldehyde. Formaldehyde is expected to exist in the gas phase as an equilibrium mixture of free and hydrated forms with about a 1:1000 ratio at equimolar concentrations of water. As both the formaldehyde concentration and the atmospheric water concentrations are variables, it is best to assume a range of rate constants and half lives for formaldehyde.

Likewise, methoxymethanol in the vapor phase will react with atmospheric water to produce formaldehyde and methanol. Methanol introduced into the atmosphere, either directly from the mixture or indirectly from hydrolysis of methoxymethanol is considered to exist primarily as the free alcohol in the gas phase when combined with air containing water vapor. The experimentally derived value of the rate constant for the reaction of methanol with hydroxyl radicals is considered more accurate than the predicted value. In addition, as methanol is not as likely to form hydrates, this rate constant is not considered a dependent variable based on atmospheric water content (as is the case with formaldehyde).

Another consideration is polymeric forms of formaldehyde. Due to dilution effects, these are not anticipated to be formed in significant quantity in the vapor phase; however, sublimation of oligomeric formaldehyde from spills of commercial methoxymethanol is possible. The final APOWIN calculation indicates that hydrogen abstraction is very a favorable process for reaction of oligomeric formaldehyde with hydroxyl radical and it will only have an atmospheric half-life on the order of 2 hour. Thus, as it is expected to contribute little to the quantity of material in the air and will not contribute to an extended half-life, it can be ignored relative to atmospheric photodegradation.

In summary, the reaction rate of methoxymethanol or commercial mixtures of formaldehyde, methanol and water with atmospheric hydroxyl radical can be described by the four species listed below.

| SPECIES | Half life (12h day 1,500,000 OH molecules/cc) |
|---|---|
| Methoxymethanol | 6.1 hours |
| Formaldehyde | 15.8 hours |
| Hydroformaldehyde | 10.9 hours |
| Methanol | 11.3 hours |

As all half-lives are relatively close, the half-life of these mixtures is suffiently well characterized for the purposes of the HPV program as having a range from 6.1 to 15.8 hours

Methoxymethanol

```
AOP Program (v1.90) Results:
============================
SMILES : COCO
CHEM   : Methoxymethanol
MOL FOR: C2 H6 O2
MOL WT : 62.07
-------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS --
Hydrogen Abstraction      =20.7705 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =20.9105 E-12 cm3/molecule-sec
 HALF-LIFE =     0.512 Days (12-hr day; 1.5E6 OH/cm3)
 HALF-LIFE =     6.138 Hrs
```

```
------------------- SUMMARY (AOP v1.90): OZONE REACTION

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches

######################################################


AOP Program (v1.90) Results:
============================
SMILES : O=C
CHEM   : Formaldehyde
MOL FOR: C1 H2 O1
MOL WT : 30.03
------------ SUMMARY (AOP v1.90): HYDROXYL RADICALS-
Hydrogen Abstraction       =8.1300 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =8.1300 E-12 cm3/molecule-sec
   HALF-LIFE =     1.316 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    15.787 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Formaldehyde
  CAS Number:  000050-00-0
 Exper OH rate constant  : 9.37  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
Exper Ozone rate constant: 2.1 E-24  cm3/molecule-sec
Exper NO3 rate constant: 3.2-7.2 E-16  cm3/molecule-sec

_____________________


---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS
SMILES : OCO
CHEM   : HYDRATED FORMALDEHYDE
MOL FOR: C1 H4 O2
MOL WT : 48.04

Hydrogen Abstraction      =11.4415 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =11.7215 E-12 cm3/molecule-sec
   HALF-LIFE =     0.913 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    10.950 Hrs

----------------- SUMMARY (AOP v1.90): OZONE REACTION -

         ******  NO OZONE REACTION ESTIMATION ******
         (ONLY Olefins and Acetylenes are Estimated)
```

```
Experimental Database:  NO Structure Matches


################################################


AOP Program (v1.90) Results:
============================
SMILES : CO
CHEM   : METHANOL
MOL FOR: C1 H4 O1
MOL WT : 32.04
------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS -
Hydrogen Abstraction       =0.4760 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.1400 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =0.6160 E-12 cm3/molecule-sec
   HALF-LIFE =    17.364 Days (12-hr day; 1.5E6 OH/cm3)

----------------- SUMMARY (AOP v1.90): OZONE REACTION --

        ******  NO OZONE REACTION ESTIMATION ******
        (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Methanol
  CAS Number:  000067-56-1
Exper OH rate constant :0.944  E-12  cm3/molecule-sec
    Exper OH Reference:  KWOK,ESC & ATKINSON,R (1994)
  Exper Ozone rate constant: --- cm3/molecule-sec
  Exper NO3 rate constant  : --- cm3/molecule-sec

   HALF-LIFE =   11.33 Days (12-hr day; 1.5E6 OH/cm3)

###############################################

SMILES : OCOCOCOCOCOCOCOCO
CHEM   : Polyformaldehyde
MOL FOR: C8 H18 O9
MOL WT : 258.23
------------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ---
Hydrogen Abstraction      =60.3924 E-12 cm3/molecule-sec
Reaction with N, S and -OH =0.2800 E-12 cm3/molecule-sec
Addition to Triple Bonds   =0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings    =0.0000 E-12 cm3/molecule-sec

 OVERALL OH Rate Constant =60.6724 E-12 cm3/molecule-sec
   HALF-LIFE =     0.176 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =     2.115 Hrs
--------------- SUMMARY (AOP v1.90): OZONE REACTION ---

          ******  NO OZONE REACTION ESTIMATION ******
          (ONLY Olefins and Acetylenes are Estimated)

Experimental Database:  NO Structure Matches
```

**Test substance :**

Methoxymethanol CASNO 4461-52-3, assumed pure

**Conclusion** :
All half-lives are relatively close, the half-life of these mixtures has a range from 6.1 to 15.8 hours regarding indirect phtolysis in the atmosphere.

**Reliability** : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

**Flag** : Critical study for SIDS endpoint

23.11.2003 (4)

### 3.1.2 STABILITY IN WATER

**Type** : abiotic
**t1/2 pH4** : = 6 minute(s) at 25 °C
**t1/2 pH7** : = 6 minute(s) at 25 °C
**t1/2 pH9** : = .5 minute(s) at 25 °C
**t1/2 pH 2** : = 2 minute(s) at 25 °C
**Deg. product** : yes
**Method** : other: chemical kinetics
**Year** :
**GLP** :
**Test substance** :

**Method** :
The rate of decomposition of methoxymethanol was measured by spectroscopically following the trapping of hydrazine derivatives of formaldehyde hydrolysis product. Determinations were made at different pH levels by recording the change in absorbance against time as a function of pH. These data were used to determine the second order rate constants for hydrolysis of methoxymethanol by water, hydrated protons and hydroxyl ion.

Estimates of hydrolysis rates as a function of pH were made by converting the second order rate constants for the hydrolysis into pseudo first-order rate constants at various pH values and estimating the half-life assuming constant water concentration and pH during the hydrolysis and using the usual relationship between first-order rate constants and half-life.

**Result** :
The second order rate constants derived for the hydrolysis are:

Reaction with water: k(w) = 3.27 E-5 M^-1 sec^-1
Reaction with H+: k(H) = 0.58
Reaction with OH-: k(OH) = 2.34 E3

Converting these to pseudo-first order rate constants and extrapolation half-lives the following t1/2 are obtained:

| | half-life | | | | | |
|---|---|---|---|---|---|---|
| Rxn with | 2 | 4 | 6 | 7 | 8 | 9 |
| Water | 6 min | 6 min | 6 min | 6 min | 6 min | 6 min |
| Acid | 2 min | 3.3 hr | 333hr | >1000hr | >1000 hr | >1000 |
| Base | >1hr | >1hr | 490min | 49 min | 4.9 min | 30 sec |

**Test substance** :
Methoxymethanol CASNO 4461-52-3, assumed pure

:
Methoxymethanol has a maximum half-life in water of 6 minutes at 25°C. Its pH dependency displays a broad peak from about pH 3 to pH 8. Above

| | | |
|---|---|---|
| **Conclusion** | | or below this range of pH the reaction with acid or base predominates over an already facile reaction with water producing and even shorter half-life. Reaction with base is faster than reaction with acids. |
| **Reliability** | : | (1) valid without restriction<br><br>Calculated from peer-reviewed experimental chemical reaction rate constants. |
| **Flag** | : | Critical study for SIDS endpoint |
| 23.12.2003 | | (7) |
| **Type** | : | abiotic |
| **t1/2 pH4** | : | at °C |
| **t1/2 pH7** | : | at °C |
| **t1/2 pH9** | : | at °C |
| **Result** | : | This preparation as typically sold, transported and used is an equilibrium mixture of formaldehyde:methanol:water in a mole ratio of about 3.3:2.0:1.0. The chemical makeup of this mixture is such that there is formally an excess of formaldehyde; however it exists primarily as a series of methanol hemiacetals and hydrates. When added to water, the equilibrium shifts rapidly toward formaldehyde hydrates and methanol.<br><br>Methanol is a simple alcohol and alcohols are one of the chemical groups considered stable to hydrolysis (Harris, 1990).<br><br>Formaldehyde is known to be water reactive reversibly forming a hydrate (HO-CH2-OH) the equilibrium constant for formaldehyde hydrate formation is > 1000 (Vollhardt, 1987). Thus, formaldehyde is known to be stable indefinitely in water, existing 99.9% as a hydrated species.<br><br>Harris, J.C. in Lyman W., Reehl, W. and Rosenblat, D. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington D.C. 1990, page 7-6<br><br>Vollhardt, Peter (1987) Organic Chemistry WH Freeman publisher NY p 637 |
| **Test substance** | : | Formcel, Celanese Chemicals' name for Methoxymethanol CASNO 4461-52-3, nominally composed of 54.5-55.5% Formaldehyde. 34.5-35.5% Methanol and 9-11% Water. |
| **Reliability** | : | (2) valid with restrictions<br><br>Estimated values based on sound chemical principles are assigned a reliability of 2. |
| **Flag** | : | Critical study for SIDS endpoint |
| 23.12.2003 | | (11) (18) |

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

### 3.3.2 DISTRIBUTION

**Media** : air - biota - sediment(s) - soil - water
**Method** : Calculation according Mackay, Level III
**Year** :

**Method** :

Since this mixture contains methoxymethanol, formaldehyde and methanol, and since the initial concentration of methoxymethanol will be readily converted to formaldehyde and methanol the calculations had to be conducted independently.

The actual physical properties for formaldehyde and methanol were input while they were allowed to be calculated for pure methoxymethanol (as they are not known with accuracy). EPIWIN was allowed to set the values for half-lives in various media. Emissions were set to equal values for air water and soil (the EPIWIN default) for consistency.

SMILES inputs
COCO
CO
C=O

**Result** :

The calculations indicate that all three major components distribute primarily to water followed closely by soil. Only methanol indicates that it we distribute to air more than a few percent. As this is a variable mixture in actual production and use, and as these materials have high water solubility and biodegradability these estimates are adequate to understand the approximate distribution of the material in the environment.

Level III Fugacity Model (Full-Output):

Chem Name : Methoxymethanol
Molecular Wt: 62.07
Henry's LC : 1.47e-006 atm-m3/mole (Henrywin program)
Vapor Press : 32 mm Hg (Mpbpwin program)
Log Kow : -1.4 (Kowwin program)
Soil Koc : 0.0163 (calc by model)

| | Concentrat (percen) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 1.92 | 12.3 | 1000 |
| Water | 54.8 | 360 | 1000 |
| Soil | 43.2 | 360 | 1000 |
| Sediment | 0.0913 | 1440 | 0 |

| | Fugacity (atm) | React (kg/h) | Advect (kg/h) | Reaction (percent) | Advection (percent) |
|---|---|---|---|---|---|
| Air | 6.12e-011 | 878 | 155 | 29.3 | 5.18 |
| Water | 5.24e-011 | 852 | 442 | 28.4 | 14.7 |
| Soil | 1.53e-009 | 672 | 0 | 22.4 | 0 |
| Sediment | 4.36e-011 | .355 | .0147 | .0118 | .000492 |

Persistence Time: 269 hr
Reaction Time: 336 hr
Advection Time: 1.35e+003 hr
Percent Reacted: 80.1
Percent Advected: 19.9

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):

Air: 12.28
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 3.213 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

METHANOL

Level III Fugacity Model (Full-Output):
==========

Chem Name : methanol
Molecular Wt: 32.04
Henry's LC : 4.55e-006 atm-m3/mole (Henry database)
Vapor Press : 127 mm Hg (user-entered)
Log Kow : -0.77 (Kowwin program)
Soil Koc : 0.0696 (calc by model)

| | Concentration (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 13 | 272 | 1000 |
| Water | 47.2 | 208 | 1000 |
| Soil | 39.7 | 208 | 1000 |
| Sediment | 0.0705 | 832 | 0 |

| | Fugacity (atm) | Reaction (kg/hr) | Advection (kg/hr) | Reaction (percent) | Advection (percent) |
|---|---|---|---|---|---|
| Air | 5.96e-010 | 199 | 782 | 6.64 | 26.1 |
| Water | 2.01e-010 | 943 | 283 | 31.4 | 9.44 |
| Soil | 6.22e-009 | 792 | 0 | 26.4 | 0 |
| Sediment | 1.5e-010 | .352 | .00846 | 0.0117 | 0.000282 |

Persistence Time: 200 hr
Reaction Time: 310 hr
Advection Time: 563 hr
Percent Reacted: 64.5
Percent Advected: 35.5

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 271.9
Water: 208.1
Soil: 208.1
Sediment: 832.3
Biowin estimate: 3.288 (days-weeks )

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

FORMALDEHYDE

Level III Fugacity Model (Full-Output):
==========

Chem Name : Formaldehyde
Molecular Wt: 30.03
Henry's LC : 3.37e-007 atm-m3/mole (Henry database)
Vapor Press : 3.89e+003 mm Hg (user-entered)
Liquid VP : 2.04e+004 mm Hg (super-cooled)
Melting Pt : 97.8 deg C (user-entered)
Log Kow : 0.35 (user-entered)
Soil Koc : 0.918 (calc by model)

| | Concentration (percent) | Half-Life (hr) | Emissions (kg/hr) |
|---|---|---|---|
| Air | 2.7 | 27.4 | 1000 |

| | | | | |
|---|---|---|---|---|
| Water | 51.3 | 360 | 1000 | |
| Soil | 45.9 | 360 | 1000 | |
| Sediment | 0.0871 | 1.44e+003 | 0 | |

| | Fugacity (atm) | Reaction (kg/hr) | Advection (kg/hr) | Reaction (percent) | Advection (percent) |
|---|---|---|---|---|---|
| Air | 1.98e-010 | 614 | 243 | 20.5 | 8.09 |
| Water | 2.59e-011 | 887 | 461 | 29.6 | 15.4 |
| Soil | 7.99e-010 | 795 | 0 | 26.5 | 0 |
| Sediment | 2.15e-011 | 0.377 | 0.0157 | 0.0126 | 0.000522 |

Persistence Time: 300 hr
Reaction Time: 391 hr
Advection Time: 1.28e+003 hr
Percent Reacted: 76.5
Percent Advected: 23.5

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 27.41
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 3.155 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

**Test substance** :

Methoxymethanol CASNO 4461-52-3, assumed pure

**Reliability** : (2) valid with restrictions

EPIWIN calculated values are assigned a reliability of 2.

**Flag** : Critical study for SIDS endpoint

22.11.2003 (6)

## 3.5 BIODEGRADATION

**Type** : aerobic
**Inoculum** : other: not pre-acclimated inoculum
**Contact time** :
**Degradation** : = 90 (±) % after 28 day(s)
**Result** : readily biodegradable
**Deg. product** :
**Method** : OECD Guide-line 301 D "Ready Biodegradability: Closed Bottle Test"
**Year** : 1990
**GLP** : no
**Test substance** : other TS

**Remark** :

Result adopted from SIDS 2003 document. Material was agreed to be readily biodegradable at the SIAM meeting

**Test substance** :

Formaldehyde CASNO 50-00-0

**Reliability** : (2) valid with restrictions
**Flag** : Critical study for SIDS endpoint

22.11.2003 (10)

**Type** : aerobic
**Inoculum** : activated sludge, domestic, non-adapted
**Contact time** :

| | | |
|---|---|---|
| **Degradation** | : | = 50 - 80 (±) % after 6 day(s) |
| **Result** | : | |
| **Remark** | : | This robust summary was adopted from the Methanol HPV document.<br><br>Please see the Methanol HPV document for additional studies.<br><br>Methanol has been well studied in biodegradation assays of several types and the weight of evidence indicates it is readily biodegradable. |
| **Test substance** | : | Methanol |
| **Reliability** | : | (2) valid with restrictions |
| **Flag** | : | Critical study for SIDS endpoint |

22.11.2003 (15)

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

| | | |
|---|---|---|
| **Type** | : | flow through |
| **Species** | : | Ictalurus melas (Fish, fresh water) |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | = 24.8 measured/nominal |
| **Limit test** | : | |
| **Analytical monitoring** | : | no |
| **Method** | : | other: acute toxicity test; "flow through bioassay" |
| **Year** | : | 1977 |
| **GLP** | : | no |
| **Test substance** | : | other TS: formalin, commercial grade, 37% |

**Method** :

fingerling; pH 6.5, water hardness 8, water temperature 12 degrees Centigrade

**Remark** :

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

**Result** :

Test result: 62.1 µl/l formalin (solution 37%)

**Test substance** :

Formaldehyde CASNO 50-00-0

**Reliability** : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

**Flag** : Critical study for SIDS endpoint

23.07.2005 (1)

| | | |
|---|---|---|
| **Type** | : | other: ECOSAR Estimate |
| **Species** | : | other: freshwater fish |
| **Exposure period** | : | 96 hour(s) |
| **Unit** | : | mg/l |
| **LC50** | : | ca. calculated |
| **Method** | : | other: Estimate |
| **Year** | : | |
| **GLP** | : | |
| **Test substance** | : | |

**Method** :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the LC50 for fish. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce methanol and

formaldehyde, which is a reactive chemical that will not fit the neutral organics model. Although this estimate is not a valid way of estimating the aquatic toxicity of methoxymethanol in solution, it has utility in estimating the contribution, if any, of the methoxymethanol molecule itself to narcosis mediated toxicity.

**Remark** :

It is fully recognized that ECOSAR is not expected to give a reliable estimate for methoxymethanol because it is a labile hemiacetal that will hydrolyze. These estimates are for the propose of demonstrating that the methoxymethanol structure itself is not expected to be toxic to aquatic species using the neutral organic model.

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to fish (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to fish, formaldehyde is the species that will determine the acute toxicity of this mixture to fish. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the LC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted LC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into fish tissues due to its Kow.

**Result** :

The ECOSAR estimate (in its entirety) is presented for completeness but the LC50 for methoxymethanol is estimated at 55% (the weight percent of formaldehyde in the mixture) of the published LC50 for formaldehyde.

ECOSAR v0.99f Class(es) Found
---
Neutral Organics

| ECOSAR Class | Organism | Duration | End Pt | Predicted mg/L |
|---|---|---|---|---|
| Neutral Organic SAR: (Baseline Toxicity) | Fish | 14-day | LC50 | 76256.125 |
| Neutral Organics | Fish | 96-hr | LC50 | 72256.133 |
| Neutral Organics | Fish | 14-day | LC50 | 76256.125 |
| Neutral Organics | Daphnid | 48-hr | LC50 | 61217.387 |
| Neutral Organics | Green Algae | 96-hr | EC50 | 31468.400 |
| Neutral Organics | Fish | 30-day | ChV | 5381.146 |
| Neutral Organics | Daphnid | 16-day | EC50 | 709.373 |
| Neutral Organics | Green Algae | 96-hr | ChV | 441.037 |
| Neutral Organics | Fish (SW) | 96-hr | LC50 | 3197.973 |
| Neutral Organics | Mysid Shrimp | 96-hr | LC50 | 2.36e+005 |
| Neutral Organics | Earthworm | 14-day | LC50 | 4256.716 |

Estimate based on formaldehyde toxicity 1/55% of 24.8 = 45 mg/L for freshwater fish.

**Test substance** :

Methoxymethanol CASNO 4461-52-3, assumed pure

**Reliability** : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

23.07.2005 (5)

## 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

| | | |
|---|---|---|
| **Type** | : | other: According to OECD standard |
| **Species** | : | Daphnia pulex (Crustacea) |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | = 5.8 measured/nominal |
| **EC10** | : | = 1.9 measured/nominal |
| **EC90** | : | = 16.8 measured/nominal |
| **Limit Test** | : | no |
| **Analytical monitoring** | : | no data |
| **Method** | : | |
| **Year** | : | 1982 |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Formaldehyde |
| **Result** | : | EC50 (48 h) = 4.3 - 7.8 (confidence limit) |
| **Test condition** | : | Stock solutions were prepared according to standard methods: APHA-AWWA-WEF (1992) and Leithe (1974). Daphnids were cultured in 3-L-aquariums and beakers that were illuminated for 12 hr per day.<br><br>test temperature: 20 +/- 1 °C,<br>Total hardness: 127 (as CaO/L)<br>PH: 8.4<br>Total solids: nil<br><br>EC10, EC50 with 95% confidence limits (f value), EC90 for daphnids were calculated using probit analysis (Statistical Support Staff Computer Sciences Corporation, 1988). |

APHA-AWWA-WEF: 1992, Standard Methods for the Examination of Water and Wastewater, 18 Edition, Washington, D.C.

Leithe,W.: 1974, Die Analyse der Luft und ihrer Verunreinigungen in der freien Atmosph¨are und am Arbeitsplatz, Wissenschaftliche Verlagsgesellschaft MBH, Stuttgart.

| | | |
|---|---|---|
| **Test substance** | : | Formaldehyde 37 % v/v |
| **Reliability** | : | (2) valid with restrictions<br><br>acceptable study, meets basic scientific principles |
| **Flag** | : | Critical study for SIDS endpoint |

23.07.2005 (17)

| | | |
|---|---|---|
| **Type** | : | other: estimate |
| **Species** | : | other: freshwater invertebrate |
| **Exposure period** | : | 48 hour(s) |
| **Unit** | : | mg/l |
| **EC50** | : | ca. 10.5 calculated |
| **Method** | : | other: Estimate |

**Year** :
**GLP** :
**Test substance** :

**Method** :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for daphnia. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

**Remark** :

It is fully recognized that ECOSAR is not expected to give a reliable estimate for methoxymethanol because it is a labile hemiacetal that will hydrolyze. These estimates are for the propose of demonstrating that the methoxymethanol structure itself is not expected to be toxic to aquatic species using the neutral organic model.

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to invertebrates (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to daphnids, formaldehyde is the species that will determine the acute toxicity of this mixture to invertebrates. In recognition of this, the robust summary flagged as "critical for SIDS endpoint" has been adopted from the formaldehyde SIDS document. The reader is referred to the formaldehyde SIDS document for more supporting studies.

The critical study was amongst the lowest of the EC50 values, and while it is recognized that there is a possibility that there will synergetic interactions between formaldehyde, this predicted EC50 is considered conservative as it was from a highly sensitive species. Significant synergism between formaldehyde and methanol is considered unlikely, as formaldehyde is a metabolic product of methanol and methanol will not distribute strongly into invertebrates tissues due to its Kow.

**Result** :

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the published EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism    Duration End Pt       mg/L
==================== =========== ======== ====  ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
Neutral Organics   : Green Algae   96-hr   EC50  31468.400
Neutral Organics   : Fish          30-day  ChV    5381.146
Neutral Organics   : Daphnid       16-day  EC50    709.373
Neutral Organics   : Green Algae   96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)    96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp  96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm     14-day  LC50   4256.716
```

Estimate based on formaldehyde toxicity 1/55% of 5.8 = 10.5 mg/L for daphnids.

**Test substance** :

Methoxymethanol CASNO 4461-52-3, assumed pure

**Reliability** : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

23.07.2005 (5)

## 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

| | | |
|---|---|---|
| **Species** | : | Scenedesmus quadricauda (Algae) |
| **Endpoint** | : | other: Oxygen uptake and prodution |
| **Exposure period** | : | 24 hour(s) |
| **Unit** | : | mg/l |
| **EC10** | : | = 3.6 measured/nominal |
| **EC50** | : | = 14.7 measured/nominal |
| **EC90** | : | = 60.3 measured/nominal |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | other TS: Formaldehyde |

**Method** :

Toxicity to algae was evaluated by measuring the oxygen production and consumption rates following exposure to the test media and calculating the 24-hr net assimilation by the algae.

The oxygen production and consumption rates were measured on Warburg apparatus (type 85G, B.Braun, Germany)

The effective concentrations were calculated using linear regression analysis.

**Remark** :

Although this was a shorter duration study, formaldehyde is so reactive that all the formaldehyde (and methylene glycol) probably reacts with algae in the first few hours of the study and a longer duration study would not be more informative.

**Test condition** :

test temperature 20 +/- 1 °C,
Standard stock solutions were prepared according to Standard Methods: APHA-AWWA-WEF, 1992 and Leithe, 1974,cultured in the nutrient solution prepared according to Holm Hansen (Bringmann and Kühn, 1980) under continuous illumination (3000 lx)

**Test substance** :

Formaldehyde (37% solution in water)

**Reliability** : (2) valid with restrictions

accepatable study, meets basic scientific principles

**Flag** : Critical study for SIDS endpoint

23.07.2005 (16)

**Species** : Scenedesmus quadricauda (Algae)
**Endpoint** : biomass

| | | |
|---|---|---|
| **Exposure period** | : | 192 hour(s) |
| **Unit** | : | mg/l |
| **EC03** | : | = .88 measured/nominal |
| **Method** | : | other: Static Cell Multiplication Inhibition Test |
| **Year** | : | 1978 |
| **GLP** | : | no |
| **Test substance** | : | other TS: Formaldehyde |

**Result** :

Toxicity Threshold : 2.5 mg/l 35% formalin
0.88 mg/l Formaldehyde

Toxic threshold is defined in this investigation as the concentration of test substance causing 3 % inhibition of cell multiplication compared to untreated controls.

**Test condition** :

Test vessel: Kapsenberg cultivation tubes (18 x 180mm)

Concentration of stock solution: not indicated

Pre-treatment of test solution: neutralisation if necessary

Inoculum:cell density adjusted to TE/F = 20 formazin turbidity equivalents at 578nm)

Test volume: 10 ml

Dilution:1:2

Number of test replicates:3

Number of control replicates:1

Illumination:constant artifical light (Osram L 40/30)

Temperature: 27 °C

Agitation: once daily

Measurements:photometric determination of cell density at578 nm after 192 h of exposure

**Test substance** :

Formalin (35% solution)

**Reliability** : (2) valid with restrictions

Test procedure in accordance with generally accepted scientific standards and described in sufficient detail

23.07.2005 (2)

| | | |
|---|---|---|
| **Species** | : | other algae: green generic |
| **Endpoint** | : | biomass |
| **Exposure period** | : | 72 hour(s) |
| **Unit** | : | mg/l |
| **EC10** | : | = calculated |
| **Method** | : | other: Estimation |
| **Year** | : | 2003 |

**GLP** :
**Test substance** :

**Remark** :

It is fully recognized that ECOSAR is not expected to give a reliable estimate for methoxymethanol because it is a labile hemiacetal that will hydrolyze. These estimates are for the propose of demonstrating that the methoxymethanol structure itself is not expected to be toxic to aquatic species using the neutral organic model.

As formaldehyde is the major component of methoxymethanol as sold, and as methanol has low acute toxicity to algae (see methanol US EPA HPV document), and as methoxymethanol itself is predicted by ESOSAR to have low toxicity to algae, formaldehyde is the species that will determine the acute toxicity of this mixture to aquatic plants. In recognition of this, two robust summaries flagged as "critical for SIDS endpoint" have been adopted from the formaldehyde SIDS document for use in the estimation of methoxymethanol toxicity to aquatic plants. The reader is referred to the formaldehyde SIDS document for additional supporting studies.

The critical studies were a long duration (192 hour) and a short duration (24 hour) study using the same species. Different endpoints were used and the estimated toxicity of methoxymethanol was calculated by taking the geometric mean of the toxic threshold value from the 192-hour study and the EC50 of the 24-hour study and setting this as the 72-hour EC50 for formaldehyde. Although there is no known scientific precedent for this calculation, it recognizes that the true value of the 72-hour EC50 for formaldehyde is lower than the 24-hour EC50. It is also recognized that formaldehyde undoubtedly reacted with the algae reducing its concentration greatly in the 192-hour study and probably in the 24-hour study. These data are considered acceptable for the estimate as ODED has recently accepted this data set for formaldehyde and as it would be impossible to accurately determine the EC50 of formaldehyde due to its reactivity and volatility.

**Result** :

The SMILES formula for methoxymethanol (COCO) was entered into ECOSAR (via EPIWIN 3.05). The program calculated critical physical properties and applies them to the neutral organic model to estimate the EC50 for algae. This was further evaluated for reasonableness and it was determined to be reasonable on chemical grounds. It was recognized, however, that hydrolysis of methoxymethanol will produce formaldehyde, which is a reactive chemical that will not fit the neutral organics model.

The ECOSAR estimate (in its entirety) is presented for completeness but the EC50 for methoxymethanol is estimated at 1/55% (the weight percent of formaldehyde in the mixture) of the calculated EC50 for formaldehyde.

```
ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
                                                        Predicted
ECOSAR Class          Organism   Duration End Pt   mg/L
==================== =========== ======== ====   ==========
Neutral Organic SAR: Fish          14-day  LC50  76256.125
(Baseline Toxicity)

Neutral Organics   : Fish          96-hr   LC50  72256.133
Neutral Organics   : Fish          14-day  LC50  76256.125
Neutral Organics   : Daphnid       48-hr   LC50  61217.387
```

```
Neutral Organics   : Green Algae  96-hr   EC50  31468.400
Neutral Organics   : Fish         30-day  ChV    5381.146
Neutral Organics   : Daphnid      16-day  EC50    709.373
Neutral Organics   : Green Algae  96-hr   ChV     441.037
Neutral Organics   : Fish  (SW)   96-hr   LC50   3197.973
Neutral Organics   : Mysid Shrimp 96-hr   LC50   2.36e+005
Neutral Organics   : Earthworm    14-day  LC50   4256.716
```

Estimate based on formaldehyde toxicity 1/55% of 6.0 (geometric mean of 196-hour EC03 and 24-hour EC50) = 11.5 mg/L for green algae.

**Test substance** :

Methoxymethanol CASNO 4461-52-3, assumed pure

**Reliability** : (2) valid with restrictions

Based on toxic component. Considered an acceptable scientific method to conduct estimate

24.07.2005 (5)

## 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

### 5.1.1 ACUTE ORAL TOXICITY

| | | |
|---|---|---|
| **Type** | : | LD50 |
| **Value** | : | = 1269 mg/kg bw |
| **Species** | : | rat |
| **Strain** | : | Crj: CD(SD) |
| **Sex** | : | male/female |
| **Number of animals** | : | |
| **Vehicle** | : | water |
| **Doses** | : | 707, 1000, 1414, 2000, 2828 |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | yes |
| **Test substance** | : | |

**Method** :

Specific guideline not specified.

The test substance in distilled water (dissolved just before administration) was administered by gavage to groups of five rats of each sex that had been fasted overnight. Doses, based on a range-finding study, were 707, 1000, 1414, 2000, and 2828 mg/kg. The volume of administration was 10 ml/kg and feed was not given for approximately three hours after administration. Purity was not determined when the test solution was prepared.

General conditions of animals were observed on the day of administration at 5 minutes, 15 minutes, 30 minutes, 1 hour, 3 and 6 hours after dosing, and once a day for a period of 14 days. Body weight was measured just before treatment, and on days 3, 7 and 14. Dead animals were necropsied promptly after discovery. After the 14-day observation period, surviving animals were sacrificed and examined. The LD50 was computed using the probit method.

**Result** :

Mortality was observed as indicated in the table below:

| Dose | MORTALITY | |
|---|---|---|
| | Males | Females |
| 0 | 0/5 | 0/5 |
| 707 | 0/5 | 0/5 |
| 1000 | 1/5 | 1/5 |
| 1414 | 3/5 | 2/5 |
| 2000 | 5/5 | 4/5 |
| 2828 | 5/5 | 5/5 |

Most deaths were within an hour of administration

LD50 values were 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg), and 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

Clinical Observations: Reduced spontaneous activity, slow breathing and blepharoptosis were observed across all groups; groups at 2000 mg/kg and

above showed lying down, gasping and clonic seizures. Salivation was reported among all groups other than the female 707 and 1414 mg/kg groups. Other symptoms included lacrimation, red lacrimation, red nasal drainage and raising of the tail.

Body Weights: Some of the surviving animals in 1414 mg/kg and 2000 mg/kg groups showed weight loss on the third day post-administration, but gained weight thereafter. Surviving animals of the other groups gained weight throughout the period of observation.

Necropsy: Animals dying from treatment showed atrial enlargement, pulmonary congestion/edema, and congestion/ edema/hemorrhaging/erosion of glandular stomach mucous membrane. Among surviving animals, adhesions of stomach and liver, thickening of the anterior stomach mucous membrane and erosion/ulceration of glandular stomach mucus membrane were noted in the groups at dose levels of 2000 and 2828 mg/kg.

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Conclusion** :

The LD50 values were:

Males: 1269 mg/kg for males (95% confidence limit: 981-1636 mg/kg)
Females: 1451 mg/kg for females (95% confidence limit: 1059-2000 mg/kg)

No specific target organs were identified.

**Reliability** : (1) valid without restriction
Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

21.08.2003 (14)

### 5.1.2 ACUTE INHALATION TOXICITY

### 5.1.3 ACUTE DERMAL TOXICITY

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

### 5.4 REPEATED DOSE TOXICITY

| | | |
|---|---|---|
| **Type** | : | Sub-acute |
| **Species** | : | rat |
| **Sex** | : | male/female |
| **Strain** | : | Crj: CD(SD) |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | 41 to 47 days |
| **Frequency of treatm.** | : | daily |
| **Post exposure period** | : | none |
| **Doses** | : | 12, 60 or 300 mg/kg-day |
| **Control group** | : | yes, concurrent vehicle |

**Method** : other: OECD Guideline 422
**Year** :
**GLP** : yes
**Test substance** :

**Method** :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5µm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation

index and the delivery index were calculated.

Organs Examined: The study report did not provide a list of organs examined; however, it was specified that the OECD 422 protocol was followed. As this protocol is specifically designed to provide an evaluation of reproductive and developmental endpoints, it can reasonably be assumed that a full range or reproductive and developmentally related organs were examined.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

**Result** :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities

and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Attached document** : Organ Wts.bmp
Hematol-ps.bmp
Biochem-ps2.bmp
Histopath.bmp

Table 3 Absolute and relative organ weight of rats treated orally with methoxymethanol in combined repeat dose and reproductive/ developmental toxicity screening test

| Sex | Dose level | 0 mg/kg | 12 mg/kg | 60 mg/kg | 300 mg/kg |
|---|---|---|---|---|---|
| Male | | | | | |
| | No. of animals | 10 | 10 | 10 | 9 |
| | Body weight (g) | 445 ± 26.1 | 440 ± 25.8 | 449 ± 32.5 | 393 ± 45.0* |
| | Absolute organ weight | | | | |
| | Thymus (mg) | 358 ± 60.0 | 415 ± 80.7 | 335 ± 44.3 | 287 ± 90.9 |
| | Liver (g) | 12.27 ± 1.547 | 11.89 ± 1.397 | 12.21 ± 1.373 | 11.45 ± 1.195 |
| | Kidneys (g) | 2.86 ± 0.250 | 2.93 ± 0.305 | 2.82 ± 0.236 | 2.72 ± 0.269 |
| | Testes (g) | 3.39 ± 0.227 | 3.15 ± 0.147 | 3.21 ± 0.344 | 3.27 ± 0.277 |
| | Epididymides (g) | 1.26 ± 0.121 | 1.22 ± 0.097 | 1.25 ± 0.127 | 1.23 ± 0.120 |
| | Relative organ weight | | | | |
| | Thymus (mg%) | 80 ± 10.7 | 95 ± 19.0 | 75 ± 9.0 | 72 ± 19.3 |
| | Liver (g%) | 2.75 ± 0.213 | 2.70 ± 0.209 | 2.71 ± 0.133 | 2.92 ± 0.233 |
| | Kidneys (g%) | 0.64 ± 0.028 | 0.67 ± 0.057 | 0.63 ± 0.041 | 0.69 ± 0.046 |
| | Testes (g%) | 0.75 ± 0.031 | 0.72 ± 0.056 | 0.72 ± 0.049 | 0.84 ± 0.112 |
| | Epididymides (g%) | 0.28 ± 0.025 | 0.28 ± 0.024 | 0.28 ± 0.018 | 0.32 ± 0.026 |
| Female | | | | | |
| | No. of animals | 10 | 10 | 10 | 9 |
| | Body weight (g) | 313 ± 14.8 | 315 ± 22.4 | 312 ± 19.7 | 310 ± 14.8 |
| | Absolute organ weight | | | | |
| | Thymus (mg) | 199 ± 69.2 | 216 ± 71.0 | 236 ± 101.8 | 185 ± 31.9 |
| | Liver (g) | 14.25 ± 0.945 | 13.84 ± 1.876 | 13.83 ± 0.567 | 15.10 ± 1.477 |
| | Kidneys (g) | 2.10 ± 0.255 | 2.11 ± 0.249 | 2.29 ± 0.574 | 2.01 ± 0.130 |
| | Relative organ weight | | | | |
| | Thymus (mg%) | 63 ± 21.2 | 69 ± 24.2 | 75 ± 29.0 | 60 ± 9.6 |
| | Liver (g%) | 4.55 ± 0.255 | 4.38 ± 0.370 | 4.45 ± 0.320 | 4.87 ± 0.446 |
| | Kidneys (g%) | 0.67 ± 0.037 | 0.67 ± 0.067 | 0.71 ± 0.176 | 0.65 ± 0.041 |

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05.

Table 1 Hematology of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

| Dose level | 0 mg/kg | 12 mg/kg | 60 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| No. of aminals | 10 | 10 | 10 | 9 |
| RBC ($\times 10^4$/mm$^3$) | 813 ± 6.0 | 815 ± 41.7 | 820 ± 24.7 | 755 ± 52.0* |
| Hematocrit (%) | 43.7 ± 1.00 | 43.6 ± 1.16 | 43.6 ± 1.18 | 38.7 ± 4.66** |
| Hemoglobin (g/dl) | 15.5 ± 0.41 | 15.4 ± 0.53 | 15.6 ± 0.32 | 13.5 ± 1.92** |
| Reticulocyte (‰) | 25 ± 3.5 | 26 ± 4.4 | 26 ± 2.8 | 45 ± 18.7** |
| MCV ($\mu$m$^3$) | 53.8 ± 1.33 | 53.6 ± 1.88 | 53.1 ± 1.62 | 51.2 ± 3.92 |
| MCH (pg) | 19.1 ± 0.60 | 19.0 ± 0.71 | 19.0 ± 0.38 | 17.8 ± 1.81 |
| MCHC (%) | 35.5 ± 0.43 | 35.4 ± 0.48 | 35.7 ± 0.53 | 34.7 ± 1.07 |
| Platelet ($\times 10^4$/mm$^3$) | 102.8 ± 11.02 | 103.3 ± 13.55 | 106.6 ± 17.65 | 127.4 ± 30.09** |
| WBC ($\times 10^2$/mm$^3$) | 104 ± 31.4 | 107 ± 29.8 | 104 ± 20.8 | 103 ± 33.4 |
| Differential leukocyte counts (%) | | | | |
| Lymphocytes | 78 ± 8.6 | 81 ± 6.2 | 83 ± 6.0 | 76 ± 8.5 |
| Neutrophils | | | | |
| segmented | 16 ± 7.8 | 12 ± 5.2 | 11 ± 6.0 | 19 ± 6.2 |
| band | 0 ± 0.3 | 1 ± 0.9 | 1 ± 0.8 | 1 ± 0.5 |
| Eosinophils | 1 ± 0.5 | 1 ± 0.9 | 1 ± 1.2 | 1 ± 0.7 |
| Basophils | 0 ± 0.0 | 0 ± 0.0 | 0 ± 0.0 | 0 ± 0.0 |
| Monocytes | 5 ± 1.9 | 5 ± 1.6 | 4 ± 2.0 | 4 ± 4.1 |

Values are expressed as Mean ± S.D.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 2 Blood chemistry of male rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

| Dose level | 0 mg/kg | 12 mg/kg | 60 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| No. of aminals | 10 | 10 | 10 | 9 |
| GOT (IU/l) | 83 ± 14.1 | 84 ± 13.1 | 78 ± 11.6 | 93 ± 11.8 |
| GPT (IU/l) | 27 ± 5.6 | 26 ± 3.5 | 26 ± 4.0 | 33 ± 9.7 |
| γ-GTP (IU/l) | 0 ± 0.0 | 0.1 ± 0.316 | 0 ± 0.0 | 0 ± 0.0 |
| ALP (IU/l) | 283 ± 32.6 | 245 ± 54.3 | 233 ± 50.9 | 199 ± 53.8 |
| Total bilirubin (mg/dl) | 0.11 ± 0.032 | 0.05 ± 0.053** | 0.10 ± 0.00 | 0.09 ± 0.033 |
| Urea nitrogen (mg/dl) | 18.5 ± 2.08 | 18.8 ± 2.64 | 18.7 ± 2.58 | 17.1 ± 4.06 |
| Creatinie (mg/dl) | 0.5 ± 0.03 | 0.5 ± 0.03 | 0.5 ± 0.06 | 0.4 ± 0.05 |
| Glucose (mg/dl) | 126 ± 8.1 | 128 ± 13.3 | 132 ± 13.7 | 115 ± 23.8 |
| Total chol. (mg/dl) | 75 ± 21.8 | 65 ± 14.8 | 69 ± 11.0 | 69 ± 8.9 |
| Triglyceride (g/dl) | 58 ± 28.4 | 49 ± 20.8 | 74 ± 36.1 | 64 ± 25.0 |
| Total protein (g/dl) | 6.69 ± 0.187 | 6.33 ± 0.476 | 6.46 ± 0.260 | 5.61 ± 0.312** |
| Albumin (g/dl) | 3.71 ± 0.083 | 3.61 ± 0.229 | 3.70 ± 0.110 | 3.38 ± 0.157** |
| A/G ratio | 1.25 ± 0.050 | 1.33 ± 0.074 | 1.34 ± 0.058 | 1.53 ± 0.190** |
| Ca (mg/dl) | 9.4 ± 0.22 | 9.3 ± 0.32 | 9.3 ± 0.21 | 8.9 ± 0.17** |
| Inorganic phos. (mg/dl) | 7.4 ± 0.46 | 7.6 ± 0.37 | 7.5 ± 0.45 | 7.5 ± 0.66 |
| Na (meq/l) | 144 ± 0.6 | 144 ± 1.0 | 144 ± 0.9 | 144 ± 0.8 |
| K (meq/l) | 4.5 ± 0.17 | 4.5 ± 0.25 | 4.5 ± 0.10 | 4.6 ± 0.52 |
| Cl (meq/l) | 105 ± 1.3 | 106 ± 2.0 | 105 ± 1.0 | 107 ± 1.3** |

Values are expressed as Mean ± S.D.
Significantly different from control group; **: P<0.01.

Table 5 Summary of histopathological findings in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

| Organ | Sex: | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose level (mg/kg): | 0 | 12 | 60 | 300 | 0 | 12 | 60 | 300 |
| findings | No. of animals: | 10 | 10 | 10 | 9 | 10 | 10 | 10 | 10 |
| Stomach | | | | | | | | | |
| Ulcer | | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 8 |
| Erosion | | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 2 |
| Focal regenerative change of gastric gland | | 0 | 0 | 3 | 6 | 0 | 0 | 0 | 5 |
| Inflammatory cell infiltration in submucosal layer | | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 5 |
| Duodenum | | | | | | | | | |
| Thickening of mucosa | | 0 | 0 | 0 | 6 | 0 | $ | $ | 0 |
| Adrenals | | | | | | | | | |
| Hypertrophy of zona fasciculata and zona reticularis | | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Kidenys | | | | | | | | | |
| Basophilic change of the tubular epithelium | | 0 | $ | $ | 0 | 2 | $ | 1/1 #) | 0 |
| Liver | | | | | | | | | |
| Peripheral fatty change | | 0 | $ | 1/1 | 0 | 0 | $ | $ | 0 |
| Focal necrosis | | 0 | $ | $ | 1 | 0 | $ | $ | 0 |
| Skin | | | | | | | | | |
| Erosion | | $ | 1/1 | $ | $ | $ | $ | $ | $ |

$: Not examined, #:Number of animals with lesion / Number of animals examined.

**Conclusion** :

Toxic effects related to administration of the test substance were observed primarily in the digestive tract and are considered to result primarily from the irritating property of the test substance. For males effects were seen at 60 mg/kg-day and above. For females, effects were seen only at the high dose.

Regarding hematology, changes in RBC's (reduced number), reticulocytes and platelets (increased) were only seen in the high-dose males. These effects may have been related to gastric ulceration and subsequent loss of blood.

Regarding clinical chemistry, effects were found only for the high-dose males. The reduction in total protein and albumin and the albumin/globulin ratio are also consistent with gastric ulceration and subsequent loss of blood.

Effects appear to be primarily at the site of contact and related to the irritant properties of the test substance. The GI tract is identified as the target organ and biochemical and hematologic changes are considered secondary to gastric ulceration and subsequent loss of blood.

The following effect levels are assigned:

LOAEL
60 mg/kg-day (males)
300 mg/kg-day (females)

NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

**Reliability** : (1) valid without restriction

Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

23.07.2005 (9)

## 5.5 GENETIC TOXICITY 'IN VITRO'

| | | |
|---|---|---|
| **Type** | : | Bacterial reverse mutation assay |
| **System of testing** | : | Salmonella typhimurium TA100, TA1535, TA98, TA 1537 and E coli WP2 uvrA |
| **Test concentration** | : | Up to 625 micrograms/plate for Salmonella and 2500 micrograms/plate for E coli. |
| **Cycotoxic concentr.** | : | Salmonella 500 micrograms/plate and above<br>E coli 1500 micrograms/plate and above |
| **Metabolic activation** | : | with and without |
| **Result** | : | positive |
| **Method** | : | |
| **Year** | : | |
| **GLP** | : | no data |
| **Test substance** | : | |

**Method** :

Using the plate incorporation method, the following bacterial strains were exposed to test material in the presence and absence of S9 mix (prepared from Sprague-Dawley type male rats induced by concurrent administration of phenobarbital and 5, 6-benzoflavone):

Salmonella typhimurium TA100
Salmonella typhimurium TA1535
Escherichia coli WP2 uvrA
Salmonella typhimurium TA98
Salmonella typhimurium TA 1537

The study was a triple plate, independent repeat design. A preliminary toxicity study was conducted using five concentrations of test material from 50 to 5000 microgram per plate. The test material was determined to be cytotoxic to Salmonella at 500 micrograms per plate and above and cytotoxic to E coli at 1500 micrograms per plate and above.

Evaluation criteria were as follows: When the number of revertant colonies on the plate containing the test substance was found to be more than two times that of the negative control, and at the same time, when reproducibility or dose dependency for its increase is seen in more than one strain of bacteria by either the direct or metabolic activation method, the said test substance was determined to be mutagenic (positive) for those strains.

**Result** :

Only strains TA100 and TA98 showed increases in revertants and data are shown in this robust summary only for these two strains

The tables below show the mean of the revertants from three replicate plates

| TA100 | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| Dose | -S9 | +S9 | -S9 | +S9 |
| 0 | 129 | 134 | 123 | 121 |
| 19.53 | 96 | 140 | 141 | 130 |
| 39.06 | 98 | 154 | 193 | 183 |
| 78.12 | 116 | 165 | 338 | 369 |
| 156.2 | 199 | 238 | 228 | 264 |
| 312.5* | 175 | 129 | 16 | 96 |
| 625* | 2 | 19 | 0 | 0 |

| TA98 | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| Dose | -S9 | +S9 | -S9 | +S9 |
| 0 | 17 | 27 | 19 | 23 |
| 19.53 | 21 | 31 | 29 | 38 |
| 39.06 | 28 | 34 | 55 | 39 |
| 78.12 | 59 | 44 | 74 | 47 |
| 156.2 | 95 | 58 | 53 | 48 |
| 312.5* | 38 | 33 | 0 | 10 |
| 625* | 0 | 0 | 0 | 0 |

* = Bacterial growth inihibition

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Conclusion** :

TA100 and TA98 showed numbers of revertants and dose dependency consistent with the evaluation criteria for a positive result. The test material is considered positive for mutagenic activity in this system under these conditions.

**Reliability** : (1) valid without restriction
Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

08.07.2005 (13)

**Type** : Chromosomal aberration test
**System of testing** : Chinese hamster lung cells
**Test concentration** : 0.005 to 0.032 mg/ml
**Cycotoxic concentr.** : 0.02 or 0.032 in the presence of S9 mix
**Metabolic activation** : with and without
**Result** : Positive
**Method** :
**Year** :
**GLP** :
**Test substance** :

**Method** :

Frozen Chinese hamster lung (CHL) cells derived from Chinese hamsters (obtained February, 1988, in the fourth successive generation from Research Resource Bank (JCRB)) were thawed and used for the test within the tenth successive generation. Eagle MEM culture medium with 10% fetal calf serum was used as the growth media.

CHL cells (20,000) were seeded into 5 ml culture medium in a flask (Croning 25 cm2) and was incubated in a CO2 incubator (5% CO2) at 37?.

For the direct method, the test substance was added on the 3rd post-seeded

day and the samples were exposed to the test substance for either 24 or 48 hours. For metabolic activation with and without the presence of S9 mix, the samples were exposed for 6 hours on the 3rd post-seeded day and upon completion of the exposure they were further cultured in fresh media for an additional 18 hours.

Dilutions of test substance were freshly prepared in acetone before each use. Containers with caps were used to minimize any changes occurring from volatilization of the substance during the preparation and handling. The test substance was dissolved in the solvent and then further diluted acetone serially to obtain the desired concentrations of the test solution. The test solution was then added to the culture media at 0.5% (v/v) for all testing. Analytical measurements of the test substance in acetone dilutions were conducted and all concentration except the 1.00 mg/ml concentration were within the acceptable range (85% of the added amount). The deviation form target concentration in the 1.0 mg/ml dilution was attributed to volatility of the test material.

Cytotoxicity was determined by adding different concentrations of MM to the cultures using the direct, the indirect and the indirect with S-9 culture conditions. Growth inhibition was measured by determining the mitotic index. The concentration exerting 50% growth inhibition (50% reduction of mitotic index) was found to be 0.020 mg/ml for the direct method while the 50% inhibitory concentrations for metabolic activation with and without S-9 mix were 0.032 mg/ml and 0.019 mg/ml, respectively. The source of the S9 was not reported.

Dose selection: Based on the results from the cell growth inhibition test, the high concentrations of the test substance were determined to be 0.020 mg/ml for the direct method and 0.032 mg/ml and 0.020 mg/ml for the metabolic activation method with S9 mix and without S9 mix, respectively. Half strength of each corresponding high concentration was used as the medium concentration and 1/4 as the low concentration.

Two hours prior to the completion of incubation, Colcemid was added to the culture media so that its final concentration was approximately 0.1µg/ml. Six slides were prepared from each petri dish and were stained with 3% Giemza solution for 10 minutes.

Slides were coded and read blind. The chromosomal analysis was based on the classification by the Japan Environmental Mutagen Association, Mammalian Mutation Study (MMS) Subcommittee, and structural aberrations of chromosome or chromatid such as gaps, breaks and exchanges, as well as polyploid cells were scored. For structural aberrations, 200 cells per group and for poliploid cells, 800 metaphase cells per each group were analyzed.

Statistics analysis was conducted using Fisher's exact test to determine the significance of differences in the number of cells with chromosomal aberrations between the solvent control groups and the groups treated with the test substance, and the positive control groups. The potential of the test substance to induce chromosomal aberrations was determined based on the criteria established by Ishidate et al. where the percentage of cells with chromosomal aberrations less than 5% is considered negative, while a percentage of more than 5% and below 10% is considered equivocal and if greater than 10% it is considered positive.

**Result** :

Results of chromosomal analysis using the direct method are shown in Table 1. As the result of exposure to methoxymethanol for 24 hours, the percentage cells with chromosomal structural aberrations and polyploid cells increased significantly in a concentration dependent relation. Methoxymethanol was determined to be positive for structural aberrations. The evaluation of polyploid cells was equivocal. With the 48-hour exposure, chromosomal structural aberrations were induced in 6% of the cells (including gaps) in the high concentration group (0.020mg/ml) indicating an equivocal result. There were also significant increases in the number of polyploid cells in the low concentration group (0.005mg/ml) and in the high concentration group (0.020 mg/ml) indicating an equivocal result for the high concentration group.

Results of chromosomal analysis using metabolic activation are shown in Table 2. Following the application of methoxymethanol, the high concentration groups with 6-hour exposure with and without the presence of S9 mix revealed chromosomal aberrations (including gaps) in 16.5%- 26% of the studied cells indicating a positive result. Further, there was a significant increase in the appearance frequency of polyploid cells among the medium and high concentration groups indicating an equivocal result.

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Attached document** : CA Tab-1.bmp
CA Tab-2.bmp

Table 1 Chromosome analysis of Chinese hamster cells (CHL) continuously treated with methoxymethanol ** without S9 mix

| Group | Concent-ration (mg/ml) | Time of exposure (hr) | No. of cells analysed | No. of structural aberrations: gap | ctb | cte | csb | cse | f | mul[2] | total | Others[3] | No. of cells with aberrations: TAG | (%) | TA | (%) | Polyploid[4] (%) | Judgement[5]: SA | NA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ( 0.0) | 0 | ( 0.0) | 0.25 | | |
| Solvent[1] | 0 | 24 | 200 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | ( 0.5) | 1 | ( 0.5) | 0.13 | | |
| MOM | 0.005 | 24 | 200 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 1 | ( 0.5) | 1 | ( 0.5) | 0.13 | − | − |
| MOM | 0.010 | 24 | 200 | 0 | 3 | 14 | 0 | 0 | 0 | 0 | 17 | 1 | 10 * | ( 5.0) | 10 * | ( 5.0) | 3.13 * | ± | − |
| MOM | 0.020 | 24 | 200 | 1 | 29 | 74 | 1 | 2 | 1 | 0 | 108 | 3 | 41 * | (20.5) | 40 * | (20.0) | 5.88 * | + | ± |
| MC | 0.00005 | 24 | 200 | 3 | 25 | 50 | 3 | 4 | 0 | 0 | 85 | 1 | 59 * | (29.5) | 57 * | (28.5) | 0.13 | + | − |
| Solvent[1] | 0 | 48 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ( 0.0) | 0 | ( 0.0) | 0.13 | | |
| MOM | 0.005 | 48 | 200 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | ( 0.5) | 1 | ( 0.5) | 1.38 * | − | − |
| MOM | 0.010 | 48 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | ( 0.0) | 0 | ( 0.0) | 1.00 | − | − |
| MOM | 0.020 | 48 | 200 | 1 | 1 | 10 | 0 | 3 | 2 | 10 | 27 | 6 | 12 * | ( 6.0) | 11 * | ( 5.5) | 5.00 * | ± | ± |
| MC | 0.00005 | 48 | 200 | 4 | 21 | 53 | 2 | 3 | 16 | 0 | 99 | 8 | 59 * | (29.5) | 59 * | (29.5) | 0.38 | + | − |

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte: chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, MC : mitomycin C.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). * : Significantly different from solvent control at $p<0.05$. ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

Table 2 Chromosome analysis of Chinese hamster cells (CHL) treated with methoxymethanol ** with and without S9 mix

| Group | Concentration (mg/ml) | S9 mix | Time of exposure (hr) | No. of cells analysed | No. of structural aberrations: gap | ctb | cte | csb | cse | f | mul[2] | total | Others[3] | No. of cells with aberrations: TAG | (%) | TA | (%) | Polyploid[4] (%) | Judgement[5]: SA | NA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | | | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ( 0.0) | 0 | ( 0.0) | 0.50 | | |
| Solvent[1] | 0 | − | 6 - (18) | 200 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | ( 0.5) | 1 | ( 0.5) | 1.50 | | |
| MOM | 0.005 | − | 6 - (18) | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ( 0.0) | 0 | ( 0.0) | 1.25 | − | − |
| MOM | 0.010 | − | 6 - (18) | 200 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | ( 1.0) | 2 | ( 1.0) | 3.25 * | − | − |
| MOM | 0.020 | − | 6 - (18) | 200 | 0 | 36 | 67 | 0 | 0 | 1 | 10 | 126 | 0 | 52 * | (26.0) | 52 * | (26.0) | 2.65 * | + | − |
| CPA | 0.005 | − | 6 - (18) | 200 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 1 | 3 | ( 1.5) | 1 | ( 0.5) | 0.13 | − | − |
| Solvent[1] | 0 | + | 6 (18) | 200 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | ( 0.5) | 1 | ( 0.5) | 0.25 | | |
| MOM | 0.008 | + | 6 - (18) | 200 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 2 | ( 1.0) | 1 | ( 0.5) | 0.13 | − | − |
| MOM | 0.016 | + | 6 - (18) | 200 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 2 | ( 1.0) | 1 | ( 0.5) | 1.63 * | − | − |
| MOM | 0.032 | + | 6 - (18) | 200 | 2 | 16 | 41 | 0 | 1 | 2 | 0 | 62 | 2 | 33 * | (16.5) | 32 * | (16.0) | 5.75 * | + | ± |
| CPA | 0.005 | + | 6 (18) | 200 | 4 | 22 | 33 | 2 | 0 | 3 | 0 | 64 | 4 | 49 * | (24.5) | 45 * | (22.5) | 0.13 | + | − |

Abbreviations : gap : chromatid gap and chromosome gap, ctb : chromatid break, cte : chromatid exchange, csb : chromosome break, cse : chromosome exchange (dicentric and ring etc.), f : acentric fragment (chromatid type), mul : multiple aberrations, TAG : total no. of cells with aberrations, TA : total no. of cells with aberrations except gap, SA : structural aberration, NA : numerical aberration, CPA : cyclophosphamide.
1) Acetone was used as solvent. 2) More than ten aberrations in a cell were scored as 10 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). 6) Seven hundred and nineteen-three cells were analysed. * : Significantly different from solvent control at $p < 0.05$ ** : Purity was 46.73%, and methanol (44.93%) was contained as impurity

**Conclusion** :
Under the conditions of this study, it is concluded that methoxymethanol induces chromosomal aberrations to CHL cells in vitro.

**Reliability** : (1) valid without restriction
Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

21.08.2003 (12)

## 5.6 GENETIC TOXICITY 'IN VIVO'

## 5.7 CARCINOGENICITY

### 5.8.1 TOXICITY TO FERTILITY

**Type** : Fertility
**Species** : rat
**Sex** : male/female
**Strain** : Crj: CD(SD)
**Route of admin.** : gavage
**Exposure period** : 14 day premating to lactation day 4
**Frequency of treatm.** : daily
**Premating exposure period**
**Male** : 14 days
**Female** : 14 days
**Duration of test** :

| | | |
|---|---|---|
| **No. of generation studies** | : | 2 |
| **Doses** | : | 12, 60 or 300 mg/lg-day |
| **Control group** | : | yes, concurrent vehicle |
| **Method** | : | OECD Guide-line 422 |
| **Year** | : | |
| **GLP** | : | yes |
| **Test substance** | : | other TS: see freetext |

**Method** :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5µm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and

other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

Organs Examined: The study report did not provide a list of organs examined; however, it was specified that the OECD 422 protocol was followed. As this protocol is specifically designed to provide an evaluation of reproductive and developmental endpoints, it can reasonably be assumed that a full range or reproductive and developmentally related organs were examined.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS:

Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

**Result** :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands. Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60

mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

REPRODUCTIVE TOX: All females that copulated resulted in pregnancy and no effect of the administered test substance on either the copulation or fertility indices was recognized. Further, most of the pairs successfully mated during the first estrous stage and there were no significant differences among the pairing days. Also, no histopathological changes were found in the ova of the single female of which copulation was unconfirmed. Reproductive parameters are shown in the table.

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Attached document** : Rerpo.bmp

Table 6 Summary of reproductive performance in rats treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

| Dose level | 0 mg/kg | 12 mg/kg | 60 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| No. of animals | 10 | 10 | 10 | 10 |
| No. of pairs copulated | 10 | 10 | 10 | 10 |
| No. of pregnant females | 10 | 10 | 10 | 10 |
| Copulation index (%) [a] | 100.0 | 90.0 | 100.0 | 100.0 |
| Fertility index (%) [b] | 100.0 | 100.0 | 100.0 | 100.0 |
| Pairing days [c] (Mean ± S.D.) | 2.8 ± 1.48 | 2.4 ± 1.01 | 3.2 ± 1.62 | 2.8 ± 1.32 |
| E.S. [d] (Mean ± S.D.) | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.1 ± 0.32 | 0.0 ± 0.00 |

a) (Number of animals with successful copulation/number of animals mated) × 100
b) (Number of pregnant animals/number of animals with successful copulation) × 100
c) Days between initial pairing and detection of copulation.
d) Number of estrous stages without copulation.

**Conclusion** :

No adverse effects were seen on reproduction in this screening study.

Reproductive NOAEL 300 mg/kg-day

Parental NOAEL
12 mg/kg-day (males)
60 mg/kg-day (females)

**Reliability** : (1) valid without restriction

Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

23.07.2005 (9)

### 5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

| | | |
|---|---|---|
| **Species** | : | rat |
| **Sex** | : | male/female |
| **Strain** | : | Crj: CD(SD) |
| **Route of admin.** | : | gavage |
| **Exposure period** | : | 14 days premating to lactation day 4 |
| **Frequency of treatm.** | : | daily |
| **Duration of test** | : | |
| **Doses** | : | 12, 60 or 300 mg/kg bw-day |
| **Control group** | : | yes, concurrent vehicle |
| **NOAEL maternal tox.** | : | = 60 mg/kg bw |
| **NOAEL teratogen.** | : | = 300 mg/kg bw |
| **NOAEL Fetotoxicity** | : | = 60 mg/kg bw |
| **Result** | : | Not specific developmental toxin |
| **Method** | : | other: OECD Guideline 422 |
| **Year** | : | |
| **GLP** | : | yes |
| **Test substance** | : | other TS: see freetext |

**Method** :

Sprague-Dawley rats (Crj:CD, SPF) obtained from Charles River Laboratories, Japan were acclimated for six days before they were divided into groups of 10 animals of each sex using stratified random sampling by weight. Rats were 8 weeks old and their weight ranged from 278-309g for males and 186-215g for females at the first dosing.

The animal room used a 12-hour day light cycle and was regulated to maintain the temperature between 20-25° C, the humidity between 40-70% R.H., and ventilation at about 12 changes of air per hour. Animals were housed in polycarbonate boxes using bedding (Betachip: Charles River Laboratories, Japan). Except during breeding, when one male and one female were co-housed, animals were individually housed. After delivery, the dam and her litter were kept in the same cage during the lactation period.

Autoclaved feed (CRF-1: Oriental Yeast Co., Ltd.) and tap water that was filtered through a 5μm filter and was irradiated with ultraviolet rays were offered ad lib.

DOSE SELECTION: Dose levels of 0,12, 60 or 300 mg/kg-day were selected based on a preliminary study with dose levels of 0, 30, 100, 300 or 1000 mg/kg-day. The 1000 mg/kg-day group showed signs of overt toxicity including reduced spontaneous activity, irregular respiration, lacrimation and death. Necropsy revealed erosion or ulceration of the stomach or duodenum in the high-dose group. The 300 mg/kg-day group was reported to show salivation and changes in the stomach but these effects were considered a LOAEL and 300 mg/kg-day was selected as the high dose for the definitive study.

STUDY CONDUCT: Males were dosed for 44 days starting 14 days prior to mating and were sacrificed the day after the last dosing. Females were dosed for 41 to 47 days starting 14 days before mating, through mating and delivery, and three days of lactation. The test substance was diluted with distilled water prior to dosing and given by gavage as a single daily administration in the morning. Dosing volume was 5ml/kg calculated based

on the most current body weight measured at that time.

Rats were mated one male and one female within the same group and allowed to mate for seven days. During this period, every day in the morning, the female's vaginal mucus was collected and was microscopically examined after it was Giemsa stained. Day zero of gestation was recorded when either a vaginal plug or sperm was found in the vaginal specimen.

Pregnant females were allowed to deliver their pups naturally. Lactation day zero was defined as completion of delivery by 9:00 in the morning of day zero. Pups were allowed to nurse until lactation day 4 and observed daily during this time for general condition, lactation, nesting, cannibalism and other significant signs. Surviving dams and pups were sacrificed on lactation-day 4. Ovaries and uteri of dams were removed to count corpora lutea and implantation sites. Based on the results obtained from these examinations, the gestation period, the gestation index, the implantation index and the delivery index were calculated.

Organs Examined: The study report did not provide a list of organs examined; however, it was specified that the OECD 422 protocol was followed. As this protocol is specifically designed to provide an evaluation of reproductive and developmental endpoints, it can reasonably be assumed that a full range or reproductive and developmentally related organs were examined.

EXAMINATION OF PUPS: Dead pups, except those that were killed and eaten and unfit for examination, were fixed in a mixed solution of formaldehyde and acetic acid before being microscopically examined. Pups from each dam were separated by sex and weighed as a group of one sex on days zero and 4. External examinations, including the oral cavity, were conducted on lactation day 4. After the examination, about half of the pups from each litter were sacrificed and prepared for skeletal examination. Pups from the control group and the high-dose group were examined for skeletal abnormalities. Pups not selected for skeletal examination were submitted to visceral examinations after fixation with a mixture of formaldehyde and acetic acid. Heads from the control and high-dose groups were examined using Wilson's method and their chest and abdomen were micro-dissected to discover any visceral abnormalities. Since there was a slightly increased occurrence of patent foramen ovale in the 300 mg/kg-day group, the 60 mg/kg-day group was also examined for visceral abnormalities.

STATISTICAL METHODS: Data were tested for homogeneity using Bartlett's method and when the distribution was normal, a one-way distribution dispersion analysis was performed. Then using either Dunnett's or Scheffe's test, the mean values were compared. When the distribution was not normal, the Kruskal-Wallis test was applied before the rank sum test of either Dunnett's or Scheffe's method. Some parameters (with asterisk) were tested initially using the Kruskal-Wallis test and when there was a significant difference, the rank sum test was performed. The calculated data were tested using Fisher's direct probability method. The level of significance was set to 5%. The mean values calculated from each maternal group were used as their statistical units for the data pertaining to the newborn pups. The following are the items for the statistical analysis.

Multiple comparison tests were used with: Weight, weight gain, feed consumption, hematological tests, blood biochemistry tests, weight of

organs, paring days*, number of estrous cycles before successful copulation*, gestation period*, number of corpora lutea, number of implantation sites, implantation index*, delivery index*, number of newborn pups, weight of newborn pups, live birth index*, viability index*, and the occurrence of skeletal and visceral abnormalities among live pups*

Fisher's direct probability method was used with: Copulation index, fertility index, gestation index, and sex ratio (male/female)

**Result** :

DEATHS: One male from the 300 mg/kg-day group died on the 14th day of administration.

CLINICAL SIGNS: Slight salivation after administration of the test substance was observed in the 300 mg/kg-day group starting on the second administration day for males, and the fourth day for the females lasting and was observed for almost all animals. Some started salivating even before the dose was given and one male showed decreased spontaneous activities and gasping on the 13th day before dying the next day. One female was observed with rales starting on the 12th day of administration and lasting through the 6th day of gestation. A few males and females in the 60 mg/kg-day group also displayed salivation but this was a sporadic occurrence.

BODY WEIGHTS: Suppression of body weight gain was noted among males of the 300 mg/kg-day group from the 7th day of administration throughout the rest of the administration period. Females did not show any significant difference between controls and dosed groups throughout the periods before mating, during gestation and after delivery.

FEED CONSUMPTION: Reduced feed consumption was noted for high dose males starting on the seventh day of dosing and continuing until sacrifice. Feed consumption for other dose groups was not different from controls before mating, during gestation period and after delivery.

HEMATOLOGY: A decrease in the red blood cell count, hematocrit value and hemoglobin concentration was noted for the high dose males as well as an increase in both reticulocyte and platelet counts. The leukocyte differential count was unremarkable for all dosed groups.

BIOCHEMISTRY: A decrease in the total protein, albumin and calcium and an increase in the A/G ratio were noted in the high-dose males. Chloride was also increased in the high-dose males but the increase was very slight and is not considered toxicologically significant.

ORGAN WEIGHTS: There was no significant difference in any of the organs between the control group and the dosed groups.

GROSS EXAMINATION: Either ulceration or erosion of the gastric glands and the proventriculus mucus membrane of the stomach were noted in 3 males and 2 females in the 300 mg/kg-day group. Five males and 4 females in the high-dose group showed the formation of gastric nodules in various sizes. Six high-dose males showed an enlarged duodenum. One high-dose male showed enlarged adrenal glands. The high-dose male that died on test had an enlarged atrium, pulmonary congestion, atrophy of the thymus gland, red patches in the gastric gland mucosa and distension of the bowel.

MICROSCOPIC EXAMINATION: Changes attributed to administration of the test substance were found in the stomach, duodenum and adrenal glands.

Ulceration of the gastric glands and the mucosa of the stomach were noted in 5 males and 8 females in the 300 mg/kg-day group. The ulcerated lesions were swollen with effused inflammatory cells and granulomatous tissue, and there were even cases which had formed either large granuloma or the pathological changes had penetrated through the muscular layer. In addition, an eroded lesion of the gastric gland where only the top layer of the mucosa had been exfoliated was found in 2 males in the 60 mg/kg-day group, and also in 3 males and 2 females in the 300 mg/kg-day group. In the 300 mg/kg-day group, 9 males and 5 females showed an inflammatory cell infiltration extending to the submucosal tissue. Focal regenerative changes of the glandular epithelium of the gastric gland was seen in 3 males in the 60 mg/kg-day group, and in 6 males and 5 females in the 300 mg/kg-day group. The focal regenerative mucosa consisted of basophilic glandular epithelia different from the normal proper glandular cells. All of these changes were most frequently seen in the proventriculus and the periphery of the gastric gland border.

Hypertrophy of the duodenal mucosa was found in 6 males of the 300 mg/kg-day group. The hypertrophied mucosa consisted of deep crypts and tall villi and there was clearly a difference between the duodena of the males in this group and those of controls.

Examination of the adrenal glands revealed hypertrophy of zona fasciculata and zona reticularis in 2 males of the 300 mg/kg-day group. These two animals also showed severe ulceration of the stomach.

DEVELOPMENTAL TOX

VIABILITY: A few still births and neonatal deaths occurred in each group, but there was no significant difference between the control group and dose groups regarding the number of pups in the litter, number of live pups, sex ratio, or live birth and viability indices.

EXTERNAL EXAMINATION: No newborn pups showed any external abnormalities in any group and their general condition subsequent to their birth indicated no abnormalities attributable to the administered test substance.

PUP WEIGHTS AND WEIGH GAIN: For both males and females, the weights measured on the lactation days 0 and 4, and the weight increase between these two dates showed no significant difference between the control group and the dose groups.

SKELETAL EXAMINATION: There were no skeletal malformations found in the control or 300 mg/kg-day groups. As variations, excess hypoglossal foramen, closure of the transverse foramen of cervical vertebrae, splitting of the ossification center of vertebral tubercle of the atlas, accessory sternebra, cervical rib, 14th rib (costal vestigium) and a shortening of the 13th rib were noted. These variations were not significantly increased as compared to the control group. Further, the occurrence of accessory sternebra in the 300mg/kg-day group was marginally significant and was considered an incidental finding.

VISCERAL EXAMINATION: There was a significant increase in the

occurrence of patent foramen ovale in the 300 mg/kg-day group. In the 300 mg/kg-day group, the incidence was 10 pups from 6 litters. Control incidence was 2 pups from 2 litters. One pup form the 60 mg/kg-day group displayed this pathology. Other findings were not dose related and were considered incidental.

VISCERAL EXAMINATION OF DEAD PUPS: The number of early-death pups that were suitable for examination was 1, 3, 2, and 9 pups from the control, 12 mg/kg-day, 60 mg/kg-day, and the 300 mg/kg-day group, respectively. Among pups found dead on the day of delivery, one high-dose pup had a hydrocephalus. Among those that expired after lactation day 1, one pup each from the control group and the high-dose group showed patent ductus arteriosus, and one pup from the 12 mg/kg group revealed dilatation of the renal pelvis. As there were few findings and no dose-response relationship these effects are considered unrelated to administration of the test substance. Other findings from the animals that died on the day of delivery include, patent foramen ovale was found in one pup from the 12 mg/kg-day group and in 2 pups from the 300 mg/kg-day group. There were also 4 cases of patent ductus arteriosus in the 300 mg/kg-day group. These findings are attributed to the fact that the pups died during parturition resulting in an incomplete closure of either the foramen ovale or ductus arteriosus.

**Test substance** :

Methoxymethanol 46.74%
Methanol 44.93%
Remainder presumed water

**Attached document** : Develop-Finds.bmp
Develop.bmp

Table 8 Skeletal and visceral findings of pups (F1)from dams (F0)treated orally with methoxymethanol in combined repeat dose and reproductive/developmental toxicity screening test

| Dose level | 0 mg/kg | | 60 mg/kg | | 300 mg/kg | |
|---|---|---|---|---|---|---|
| No. of dams | 10 | | 10 | | 10 | |
| Skeletal examination | | | | | | |
| No. of pups examined | 78 | | $ | | 77 | |
| No. of abnormal pups (%) | 15 | (18.9) | | | 13 | (16.3) |
| Foramen hypoglossi double | 1 | (1.3) | | | 0 | |
| Closure of transverse foramen of one or more cervical vertebrae | 4 | (5.1) | | | 9 | (11.1) |
| Splitting of ossification centers of the ventral tubercle of the atlas | 0 | | | | 1 | (1.3) |
| Accessory sternebrae | 5 | (6.2) | | | 0 | ** |
| Cervical ribs | 0 | | | | 1 | (1.4) |
| 14th ribs | 2 | (2.5) | | | 0 | |
| Reduced 13th ribs | 4 | (5.0) | | | 2 | (2.5) |
| Visceral examination | | | | | | |
| No. of pups examined | 72 | | 62 | | 72 | |
| No. of abnormal pups (%) | 8 | (10.9) | 8 | (12.2) | 16 | (22.4) |
| Thymic remunant in the neck | 3 | (4.1) | 2 | (3.3) | 3 | (4.2) |
| Deformity of the heart | 1 | (1.4) | 0 | | 0 | |
| Patent foramen ovale | 2 | (2.7) | 1 | (1.7) | 10 | (14.5)* |
| Patent ductus arteriosus | 2 | (2.7) | 2 | (2.9) | 3 | (3.8) |
| Supernumerary of the cornary orifice | 2 | (2.7) | 0 | | 0 | |
| High take off of the cornary orifice | 0 | | 0 | | 1 | (1.3) |
| Dilatation of the renal pelvis | 0 | | 3 | (4.3)** | 0 | |

$: Not examined.
Significantly different from control group; *: P<0.05, **: P<0.01.

Table 7 Findings of delivery in dams (F0) treated orally with methoxymethanol and observation on their pups (F1) in combined repeat dose and reproductive/developmental toxicity screening test

| Dose level | | | 0 mg/kg | 12 mg/kg | 60 mg/kg | 300 mg/kg |
|---|---|---|---|---|---|---|
| No. of dams observed | | | 10 | 9 | 10 | 10 |
| No. of dams observed live pups | | | 10 | 9 | 10 | 10 |
| Gestation length | | | 22.7 ± 0.48 | 22.4 ± 0.73 | 22.4 ± 0.52 | 22.2 ± 0.42 |
| No. of corpora lutea | | | 18.1 ± 2.64 | 17.9 ± 3.92 | 18.2 ± 4.54 | 19.3 ± 4.00 |
| No. of implantation sites | | | 16.4 ± 1.35 | 14.3 ± 4.92 | 14.6 ± 3.24 | 16.4 ± 1.84 |
| No. of pups born | | | 15.2 ± 1.55 | 14.2 ± 5.07 | 13.6 ± 3.20 | 15.9 ± 1.73 |
| No. of live pups on day 0 | | | 15.2 ± 1.55 | 14.1 ± 5.04 | 13.5 ± 3.10 | 15.2 ± 1.75 |
| Male | | | 7.0 ± 2.40 | 7.1 ± 3.59 | 5.7 ± 2.35 | 6.9 ± 2.33 |
| Female | | | 8.2 ± 2.94 | 7.0 ± 3.57 | 7.8 ± 2.53 | 8.3 ± 2.21 |
| Sex ratio (Male/Female) | | | 0.85 (70/82) | 1.02 (64/63) | 0.73 (57/78) | 0.85 (69/83) |
| No. of live pups on day 4 | | | 15.0 ± 1.49 | 13.6 ± 5.34 | 13.1 ± 2.81 | 14.9 ± 1.66 |
| Male | | | 6.9 ± 2.51 | 6.9 ± 3.92 | 5.6 ± 2.22 | 6.8 ± 2.15 |
| Female | | | 8.1 ± 2.96 | 6.7 ± 3.16 | 7.5 ± 2.32 | 8.1 ± 2.02 |
| Gestation index (%) [a] | | | 100 | 100 | 100 | 100 |
| Implantation index (%) [b] | | | 91.6 ± 9.86 | 80.4 ± 24.98 | 81.9 ± 18.70 | 87.7 ± 16.05 |
| Delivery index (%) [c] | | | 92.7 ± 5.37 | 91.8 ± 16.20 | 93.0 ± 7.03 | 97.1 ± 4.00 |
| Live birth index (%) [d] | | | 100.0 ± 0.00 | 99.3 ± 2.07 | 99.4 ± 1.87 | 95.8 ± 6.35 |
| Viability index (%) [e] | | | 98.7 ± 2.66 | 86.1 ± 33.33 | 97.5 ± 4.35 | 98.1 ± 3.06 |
| Pups body weight | | | | | | |
| Male | On day | 0 | 6.9 ± 0.54 | 6.4 ± 0.55 | 6.9 ± 0.94 | 6.2 ± 0.59 |
| | | 4 | 11.1 ± 0.99 | 10.5 ± 0.84 | 10.9 ± 2.25 | 10.0 ± 0.85 |
| | Gain | 0-4 | 4.1 ± 0.59 | 4.1 ± 0.48 | 4.0 ± 1.45 | 3.8 ± 0.43 |
| Female | On day | 0 | 6.5 ± 0.60 | 6.3 ± 0.77 | 6.6 ± 0.87 | 5.9 ± 0.47 |
| | | 4 | 10.6 ± 0.95 | 9.9 ± 1.15 | 10.7 ± 2.04 | 9.5 ± 0.93 |
| | Gain | 0-4 | 4.1 ± 0.54 | 3.9 ± 0.50 | 4.0 ± 1.25 | 3.6 ± 0.58 |

a) (Number of females with live pups/number of pregnant females) ×100
b) (Number of total implants/number of total corpora lutea) ×100
c) (Number of total pups/number of total implants) ×100
d) (Number of total live pups on day 0 after birth/number of total pups born) ×100
e) (Number of total live pups on day 4 after birth/number of total live pups on day 0) ×100

**Conclusion** :

No malformations were observed that were attributable to administration of the test substance. High-dose pups were not different from controls in body weight, sex ratio, mean pup weights, number of pups born, or other similar parameters. Visceral examination revealed a significant increase in the occurrence of patent foramen ovale in the 300 mg/kg-day group. This is interpreted as a fetotoxic effect at the high dose associated with a developmental delay.

Developmental NOAEL 60 mg/kg-day

Maternal NOAEL 60 mg/kg-day

**Reliability** : (1) valid without restriction

Guideline or guideline-like study with good documentation

**Flag** : Critical study for SIDS endpoint

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